PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

PCT

To:
J.A. KEMP & CO.
Attn. WRIGHT, Simon M.
14 South Square
Gray's Inn
London WC1R 5LX
UNITED KINGDOM

J. A. KEMP & CO
REC'D 24 MAR 2000
Action by

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL SEARCH REPORT OR THE DECLARATION

(PCT Rule 44.1)

Applicant's or agent's file reference N.74544A SMW	Date of mailing (day/month/year) 21/03/2000
International application No. PCT/EP 99/07834	FOR FURTHER ACTION See paragraphs 1 and 4 below
Applicant DSM N.V et al	International filing date (day/month/year) 15/10/1999

1. [X] The applicant is hereby notified that the International Search Report has been established and is transmitted herewith.

Filing of amendments and statement under Article 19:
The applicant is entitled, if he so wishes, to amend the claims of the International Application (see Rule 46):

When? The time limit for filing such amendments is normally 2 months from the date of transmittal of the International Search Report; however, for more details, see the notes on the accompanying sheet.

Where? Directly to the International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Fascimile No.: (41-22) 740.14.35

For more detailed instructions, see the notes on the accompanying sheet.

2. [ ] The applicant is hereby notified that no International Search Report will be established and that the declaration under Article 17(2)(a) to that effect is transmitted herewith.

3. [ ] **With regard to the protest** against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:

[ ] the protest together with the decision thereon has been transmitted to the International Bureau together with the applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.

[ ] no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.

4. **Further action(s):** The applicant is reminded of the following:

Shortly after **18 months** from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau as provided in Rules 90*bis*.1 and 90*bis*.3, respectively, before the completion of the technical preparations for international publication.

Within **19 months** from the priority date, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later).

Within **20 months** from the priority date, the applicant must perform the prescribed acts for entry into the national phase before all designated Offices which have not been elected in the demand or in a later election within 19 months from the priority date or could not be elected because they are not bound by Chapter II.

Name and mailing address of the International Searching Authority	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Renate Jordan

Form PCT/ISA/220 (July 1998)

NOTES TO FORM PCT/ISA/220

These Notes are intended to give the basic instructions concerning the filing of amendments under article 19. The Notes are based on the requirements of the Patent Cooperation Treaty, the Regulations and the Administrative Instructions under that Treaty. In case of discrepancy between these Notes and those requirements, the latter are applicable. For more detailed information, see also the PCT Applicant's Guide, a publication of WIPO.

In these Notes, "Article", "Rule", and "Section" refer to the provisions of the PCT, the PCT Regulations and the PCT Administrative Instructions respectively.

INSTRUCTIONS CONCERNING AMENDMENTS UNDER ARTICLE 19

The applicant has, after having received the international search report, one opportunity to amend the claims of the international application. It should however be emphasized that, since all parts of the international application (claims, description and drawings) may be amended during the international preliminary examination procedure, there is usually no need to file amendments of the claims under Article 19 except where, e.g. the applicant wants the latter to be published for the purposes of provisional protection or has another reason for amending the claims before international pbulication. Furthermore, it should be emphasized that provisional protection is available in some States only.

What parts of the international application may be amended?

Under Article 19, only the claims may be amended.

During the international phase, the claims may also be amended (or further amended) under Article 34 before the International Preliminary Examining Authority. The description and drawings may only be amended under Article 34 before the International Examining Authority.

Upon entry into the national phase, all parts of the international application may be amended under Article 28 or, where applicable, Article 41.

When?

Within 2 months from the date of transmittal of the international search report or 16 months from the priority date, whichever time limit expires later. It should be noted, however, that the amendments will be considered as having been received on time if they are received by the International Bureau after the expiration of the applicable time limit but before the completion of the technical preparations for international publication (Rule 46.1).

Where not to file the amendments?

The amendments may only be filed with the International Bureau and not with the receiving Office or the International Searching Authority (Rule 46.2).

Where a demand for international preliminary examination has been/is filed, see below.

How?

Either by cancelling one or more entire claims, by adding one or more new claims or by amending the text of one or more of the claims as filed.

A replacement sheet must be submitted for each sheet of the claims which, on account of an amendment or amendments, differs from the sheet originally filed.

All the claims appearing on a replacement sheet must be numbered in Arabic numerals. Where a claim is cancelled, no renumbering of the other claims is required. In all cases where claims are renumbered, they must be renumbered consecutively (Administrative Instructions, Section 205(b)).

The amendments must be made in the language in which the international application is to be published.

What documents must/may accompany the amendments?

Letter (Section 205(b)):

The amendments must be submitted with a letter.

The letter will not be published with the international application and the amended claims. It should not be confused with the "Statement under Article 19(1)" (see below, under "Statement under Article 19(1)").

The letter must be in English or French, at the choice of the applicant. However, if the language of the international application is English, the letter must be in English; if the language of the international application is French, the letter must be in French.

The letter must indicate the differences between the claims as filed and the claims as amended. It must, in particular, indicate, in connection with each claim appearing in the international application (it being understood that identical indications concerning several claims may be grouped),whether

(i) the claim is unchanged;

(ii) the claim is cancelled;

(iii) the claim is new;

(iv) the claim replaces one or more claims as filed;

(v) the claim is the result of the division of a claim as filed.

The following examples illustrate the manner in which amendments must be explained in the accompanying letter:

1. [Where originally there were 48 claims and after amendment of some claims there are 51]:
"Claims 1 to 29, 31, 32, 34, 35, 37 to 48 replaced by amended claims bearing the same numbers; claims 30, 33 and 36 unchanged; new claims 49 to 51 added."

2. [Where originally there were 15 claims and after amendment of all claims there are 11]:
"Claims 1 to 15 replaced by amended claims 1 to 11."

3. [Where originally there were 14 claims and the amendments consist in cancelling some claims and in adding new claims]:
"Claims 1 to 6 and 14 unchanged; claims 7 to 13 cancelled; new claims 15, 16 and 17 added." or
"Claims 7 to 13 cancelled; new claims 15, 16 and 17 added; all other claims unchanged."

4. [Where various kinds of amendments are made]:
"Claims 1-10 unchanged; claims 11 to 13, 18 and 19 cancelled; claims 14, 15 and 16 replaced by amended claim 14; claim 17 subdivided into amended claims 15, 16 and 17; new claims 20 and 21 added."

"Statement under article 19(1)" (Rule 46.4)

The amendments may be accompanied by a statement explaining the amendments and indicating any impact that such amendments might have on the description and the drawings (which cannot be amended under Article 19(1)).

The statement will be published with the international application and the amended claims.

It must be in the language in which the international appplication is to be published.

It must be brief, not exceeding 500 words if in English or if translated into English.

It should not be confused with and does not replace the letter indicating the differences between the claims as filed and as amended. It must be filed on a separate sheet and must be identified as such by a heading, preferably by using the words "Statement under Article 19(1)."

It may not contain any disparaging comments on the international search report or the relevance of citations contained in that report. Reference to citations, relevant to a given claim, contained in the international search report may be made only in connection with an amendment of that claim.

Consequence if a demand for international preliminary examination has already been filed

If, at the time of filing any amendments under Article 19, a demand for international preliminary examination has already been submitted, the applicant must preferably, at the same time of filing the amendments with the International Bureau, also file a copy of such amendments with the International Preliminary Examining Authority (see Rule 62.2(a), first sentence).

Consequence with regard to translation of the international application for entry into the national phase

The applicant's attention is drawn to the fact that, where upon entry into the national phase, a translation of the claims as amended under Article 19 may have to be furnished to the designated/elected Offices, instead of, or in addition to, the translation of the claims as filed.

For further details on the requirements of each designated/elected Office, see Volume II of the PCT Applicant's Guide.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
N.74544A SMW		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 07834	15/10/1999	15/10/1998
Applicant		
DSM N.V et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/EP 99/07834

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/20 A23L1/30 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 37200 A (SCOTIA HOLDINGS PLC) 28 November 1996 (1996-11-28) cited in the application page 4, line 4-12; claims 4,6; examples 3-5	1,3,4,11
A	WO 92 13086 A (MARTEK CORPORATION) 6 August 1992 (1992-08-06) cited in the application	1
A	EP 0 733 360 A (SCOTIA HOLDINGS PLC) 25 September 1996 (1996-09-25) page 3, column 54-56; examples 4-6	1,5,10, 16,17, 19-21

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2000	21/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Caturla Vicente, V

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 409 559 A (EFAMOL HOLDINGS PLC) 23 January 1991 (1991-01-23) page 5, line 10-17 page 6, line 56 -page 7, line 2; claims 1-5	1-5,9, 15-22
X	WO 96 40106 A (MARTEK BIOSCIENCES CORPORATION) 19 December 1996 (1996-12-19) claims 30,31,39-41,61-64	1-7,9, 10,16, 17,19
A	DATABASE WPI Section Ch, Week 9822 Derwent Publications Ltd., London, GB; Class D13, AN 98-250984 XP002099507 & WO 98 16119 A (SUNTORY LTD), 23 April 1998 (1998-04-23) abstract	11,12
X	DATABASE WPI Section Ch, Week 9816 Derwent Publications Ltd., London, GB; Class D16, AN 98-179447 XP002099508 & WO 98 08967 A (SUNTORY LTD), 5 March 1998 (1998-03-05) abstract	14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/EP 99/07834

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9637200 A	28-11-1996	AU 5827796 A	11-12-1996
		CA 2195979 A	28-11-1996
		EP 0774962 A	28-05-1997
		FI 970298 A	24-01-1997
		JP 10503531 T	31-03-1998
		NO 970317 A	24-01-1997
		ZA 9604215 A	04-12-1996
WO 9213086 A	06-08-1992	AU 661674 B	03-08-1995
		AU 1235592 A	27-08-1992
		BR 9205519 A	01-03-1994
		CA 2101273 A	25-07-1992
		DE 568608 T	22-04-1999
		EP 0568608 A	10-11-1993
		IL 100732 A	29-06-1995
		JP 11151075 A	08-06-1999
		JP 6505384 T	23-06-1994
		MX 9200301 A	01-08-1992
		NZ 241358 A	27-09-1994
		OA 9909 A	15-09-1994
		US 5658767 A	19-08-1997
		ZA 9200454 A	28-10-1992
EP 733360 A	25-09-1996	AT 144706 T	15-11-1996
		AU 666782 B	22-02-1996
		AU 5183093 A	09-06-1994
		AU 5232996 A	18-07-1996
		CA 2109777 A	27-05-1994
		CN 1104494 A	05-07-1995
		DE 69305723 D	05-12-1996
		DE 69305723 T	03-04-1997
		DK 599576 T	25-11-1996
		EP 0599576 A	01-06-1994
		ES 2093935 T	01-01-1997
		GR 3021692 T	28-02-1997
		HK 114297 A	29-08-1997
		JP 6199663 A	19-07-1994
		NO 934266 A	27-05-1994
		NZ 250265 A	24-06-1997
		SG 47838 A	17-04-1998
		US 5516800 A	14-05-1996
		ZA 9308835 A	02-08-1994
EP 409559 A	23-01-1991	AT 116849 T	15-01-1995
		AU 625705 B	16-07-1992
		AU 5911590 A	24-01-1991
		CA 2021000 A	22-01-1991
		DE 69015910 D	23-02-1995
		DE 69015910 T	08-06-1995
		DK 409559 T	27-03-1995
		ES 2066134 T	01-03-1995
		GR 3015722 T	31-07-1995
		HK 109395 A	14-07-1995
		IE 64910 B	20-09-1995
		JP 3066616 A	22-03-1991
		NZ 234528 A	24-06-1997
WO 9640106 A	19-12-1996	AU 6252196 A	30-12-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 99/07834

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9640106 A		EP 0831805 A	01-04-1998
WO 9816119 A	23-04-1998	JP 10191886 A	28-07-1998
		AU 4471997 A	11-05-1998
		EP 0956774 A	17-11-1999
WO 9808967 A	05-03-1998	JP 10070992 A	17-03-1998
		AU 4031197 A	19-03-1998
		CN 1232507 A	20-10-1999
		EP 0957173 A	17-11-1999

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9621037	11-07-96	AU-A-	4854296	24-07-96
WO-A-9428913	22-12-94	AU-A-	6963594	03-01-95
		EP-A-	0707487	24-04-96
		JP-T-	8511533	03-12-96
EP-A-599576	01-06-94	AT-T-	144706	15-11-96
		AU-B-	666782	22-02-96
		AU-A-	5183093	09-06-94
		AU-A-	5232996	18-07-96
		CA-A-	2109777	27-05-94
		CN-A-	1104494	05-07-95
		DE-D-	69305723	05-12-96
		EP-A-	0733360	25-09-96
		ES-T-	2093935	01-01-97
		JP-A-	6199663	19-07-94
		NO-A-	934266	27-05-94
		US-A-	5516800	14-05-96
		ZA-A-	9308835	02-08-94
EP-A-342795	23-11-89	JP-A-	2049723	20-02-90
		JP-B-	2524217	14-08-96
GB-A-2218904	29-11-89	NONE		
GB-A-2221843	21-02-90	AT-B-	398779	25-01-95
		AT-A-	191889	15-06-94
		AU-B-	616784	07-11-91
		AU-A-	3896789	15-02-90
		BE-A-	1002547	19-03-91
		CA-A-	1337548	14-11-95
		CH-A-	680789	13-11-92
		DE-A-	3926658	15-02-90
		FR-A-	2635263	16-02-90
		GR-B-	1000567	26-08-92
		HK-A-	84196	24-05-96
		IE-B-	64524	09-08-95
		IL-A-	91275	19-01-96
		JP-A-	2104522	17-04-90
		LU-A-	87570	08-01-90

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

1. Claims 1-66,73-77 (completely)
   Claims 70-72,78,83-83 (partially)
   Use of a composition containing oil comprising HUFA, in particular a composition containing a single cell microbial oil comprising DHA and/or ARA for treating a neurological disorder.

2. Claims 67-69
   Use of an oil enriched in DHA for lowering triglyceride content in plasma of a patient.

3. Claims 70-72,78 (partially)
   Use of a composition containing oil comprising HUFA, in particular a composition containing oil comprising DHA for treating phenylketonuria.

4. Claims 70-72,78 (partially)
   Use of a composition containing oil comprising HUFA, in particular a composition containing oil comprising DHA for treating cystic fibrosis.

5. Claims 79-82
   Use of a composition containg oil comprising HUFA for coorecting lipid imbalance in a patient.

6. Claims 83-84 (partially)
   Use of an oil enriched in DHA for treating cardiac disorders.

Groups searched: 1,2,3 and 6
Groups not searched: 4 and 5

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 96/08649

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
GB-A-2221843		NL-A-	8902020	01-03-90
		SE-A-	8902701	12-02-90
		US-A-	5502077	26-03-96
GB-A-2148713	05-06-85	US-A-	4526902	02-07-85
		CA-A-	1239587	26-07-88
		CH-A-	661209	15-07-87
		DE-A-	3438630	02-05-85
		FR-A-	2553662	26-04-85
		JP-A-	60115522	22-06-85
		SE-B-	462701	20-08-90
		SE-A-	8405308	25-04-85
WO-A-9428891	22-12-94	AU-A-	7053494	03-01-95

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
N.74544A SMW		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 99/ 07834	15/10/1999	15/10/1998
Applicant		
DSM N.V et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

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   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

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6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
/EP 99/07834

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/20 A23L1/30 A23K1/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 37200 A (SCOTIA HOLDINGS PLC) 28 November 1996 (1996-11-28) cited in the application page 4, line 4-12; claims 4,6; examples 3-5	1,3,4,11
A	WO 92 13086 A (MARTEK CORPORATION) 6 August 1992 (1992-08-06) cited in the application	1
A	EP 0 733 360 A (SCOTIA HOLDINGS PLC) 25 September 1996 (1996-09-25) page 3, column 54-56; examples 4-6	1,5,10, 16,17, 19-21

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 March 2000	21/03/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Caturla Vicente, V

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted] International Application No
[redacted]/EP 99/07834

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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X	WO 96 40106 A (MARTEK BIOSCIENCES CORPORATION) 19 December 1996 (1996-12-19) claims 30,31,39-41,61-64	1-7,9, 10,16, 17,19
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1

INTERNATIONAL SEARCH REPORT

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International Application No
/EP 99/07834

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International Application No
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
N.74544A SMW		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/07834	15/10/1999	15/10/1998
International Patent Classification (IPC) or national classification and IPC A61K31/20		
Applicant DSM N.V et al		

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   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
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Date of submission of the demand	Date of completion of this report
06/12/1999	24.10.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Cattell, James Telephone No. +49 89 2399 8468

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/07834

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Description, pages:

1-19 as originally filed

Claims, No.:

1-22 as originally filed

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1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1,2,3,4,5,6,7,8,9,10,12,15,16,17,18,19,20, 22
Inventive step (IS)	Yes:	Claims	
	No:	Claims	11,13,14,21
Industrial applicability (IA)	Yes:	Claims	1-22
	No:	Claims	

2. Citations and explanations

see separate sheet

V.

1). Document D1 (WO96/40106) discloses in claim 40 a composition suitable for a dosage of 1g/day of ARA also containing 500 mg DHA.
This composition falls within the scope of claims 1, 3, 4,5, 9, 16, 19 and 20 under Article 33(2) PCT.

Document D2 (Ep-A-0,733,360) discloses in examples 4 to 6 a composition containing 200mg ARA and 200mg DHA.
This composition falls within the scope of claims 1, 3, 5, 6, 9, 10, 16,17, 19, 20 and 21 under Article 33(29 PCT.

Document D3 (EP-A,0409,559) discloses a capsule of 300mg ARA falling within the scope of claims 1, 2, 6, 7, 8, 9, 12(see D3 page 5 lines 5 to 18), 13, 15, 16, 18 and 22 under Article 33(2) PCT.

2). The developments of claims 11, 13 and 14 would seem obvious in the light of any one of D1 to D3. These claims therefore do not met the requirements of Article 33(3) PCT.

3). Documents D4 (WPI 98-250984) and D5 (WPI-98-179447) both discuss the use of ARA in pregnant women and nursing mothers. Claim 15 is therefore not novel under Article 33(2) PCT.

4). Claim 21 would seem obvious in the light of D3 page 5 line 12.

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To:

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NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 24.10.2000
Applicant's or agent's file reference N.74544A SMW	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP99/07834	15/10/1999	15/10/1998

Applicant
DSM N.V et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

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For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

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Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Cattell, James Telephone No. +49 89 2399 8468

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	No:	Claims	

2. Citations and explanations

see separate sheet

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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A1

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(71) Applicant *(for all designated States except US)*: DSM N.V. [NL/NL]; Het Overloon 1, NL–6411 TE Heerlen (NL).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: VAN WATERSCHOOT, Isabel, Antonia, Maria [NL/NL]; Boslaan 18, NL–8302 AB Emmeloord (NL). STREEKSTRA, Hugo [NL/NL]; Weteringstraat 28–1, NL–1017 SP Amsterdam (NL).

(74) Agent: WRIGHT, Simon, Mark; J. A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PUFA SUPPLEMENTS

(57) Abstract

Edible formulations, such as polyunsaturated fatty acids (PUFAs) such as pharmaceutical compositions or nutritional supplements, are disclosed comprising arachidonic acid (ARA). They are adapted to deliver from 150 mg to 1 g per day of ARA and may contain other PUFAs, for example docosahexaenoic acid (DHA). The DHA dosage is from 400 to 600 mg per day, and the ratio of ARA:DHA may be from 1:5 to 5:1. Pharmaceutical compositions comprising ARA and DHA at a ratio of ARA:DHA of 1:1 to 1:2 are also disclosed, as are foodstuffs comprising 0.1 to 5 % ARA. Such formulations can be used to increase ARA levels *in vivo*, for example in pregnant women or for people who have diseases or conditions associated with low ARA levels.

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PUFA SUPPLEMENTS

This invention relates to the provision of polyunsaturated fatty acids (PUFAs) in the diet of humans and animals. More specifically it relates to the provision of polyunsaturated fatty acids of the n-6 and the n-3 families, and in particular the n-6 fatty acid arachidonic acid (ARA) and the n-3 fatty acid docosahexaenoic acid (DHA), and ratios thereof in balanced amounts.

The invention is in part based on the finding that an optimal balance of the n-6 and n-3 families can play a significant role in health and the prevention of chronic diseases. The main reason for this is that the two families compete for the same enzyme(s) for the formation of the long-chain members from their C18 precursors. As a consequence, and this occurs in prior art compositions, a surplus of member(s) of one family tends to depress the amount of the other family. Moreover, the members of the two families can in some circumstances have adverse effects on essential functions in the body, such as blood clotting and the immune response.

Introduction

It is technologically relatively easy to provide the C18 n-6 fatty acid linoleic acid in the diet, since this fatty acid is abundantly present in common vegetable oils, such as corn oil and soy oil. There are also plant oils available that contain the C18 n-3 fatty acid α-linolenic acid, for instance rape seed oil, but these are much less readily used due to their lower stability. This usually leads to a surplus of the n-6 family over the n-3 family in the modern diet.

It has therefore been argued that n-3 fatty acids should be supplemented in many cases where a relative depletion is suspected. Generally this cannot be achieved by providing the C-18 precursor, since the efficiency of its conversion to C20 and C22 derivatives is low. Therefore, the consensus is that the C20 and C22 n-3 fatty acids (EPA and DHA) should be provided themselves.

In many cases the rationale behind this supplementation is to attenuate the action of the long-chain n-6 fatty acid ARA. It has been shown that the addition of the n-3 PUFAs, either derived from fish oil or from microbial (algae) oils does indeed lead to lower ARA levels. In the case of fish oil this occurs in spite of the fact that fish oil

contains low amounts of ARA.

This depression of the ARA content is not always desirable. The invention thus seeks to provide preparations that may enhance the DHA and/or EPA status of animals, without adversely affecting ARA levels, or, conversely, enhance ARA without affecting the DHA and/or EPA status.

The use of preparations containing both ARA and n-3 PUFAs has been described before in the provision of PUFAs to infant formula. The rationale behind this is that human breast milk contains appreciable amounts of ARA and DHA which are considered useful to the developing infant.

In contrast, for adult nutrition there is no such natural source of PUFAs, although both ARA and DHA can be found as components of the human diet. However, for a number of reasons these PUFA levels appear to be sub-optimal. Furthermore, different populations have different levels of these PUFAs and this can affect the suitable dosage. As there is no model from nature, the relative amounts of PUFAs to be used needs to be determined and the present invention seeks to address this problem and provide various formulations and proportions of the PUFAs for certain applications.

Prior Art

M. Makrides *et al*, European Journal of Chemical Nutrition 50:352-357 (1996) refers to a study to assess the effect of varying the internal intake of DHA (from 0 to 1.3g DHA/day) on breast milk fatty acids. DHA in the diet fed to lactating mothers had a strong specific and dose-dependent effect on breast milk DHA but did not affect ARA levels. This study used algae oils available from Martek Corporation, USA, under the brand name NEUROMINS™.

WO-A-92/12711 (Martek) refers to oil blends containing ARA and DHA, for example an ARA:DHA ratio of 3:1 to 2:1, in particular to provide levels of these PUFAs in infant formula in amounts comparable to human breast milk (which has an ARA level of 0.5 to 0.6%).

A number of PUFA-containing compositions are currently marketed. EFANATAL™ are capsules, two capsules to be taken per day to give a daily intake of DHA (125mg), ARA (8.6mg) and GLA (40mg). The capsules contain an oil that is primarily based on fish oil. The Applicant has found that this decreases *in vivo* ARA levels, because the DHA content relative to the ARA content in the capsules is too high. Thus this product is in fact an ARA lowering, rather than ARA increasing, composition

despite the fact that it contains ARA. A comparison between this product and those of the invention is provided later.

EFAMARINE™ is also capsules, containing primarily fish and evening primrose oils, of which two are to be taken per day to give a daily intake of EPA (34mg), DHA (22mg) and GLA (68mg).

EFALEX™ is an oil blend, where a teaspoon (5ml) is intended to be taken twice a day, each teaspoon giving DHA (100mg), GLA (21mg), ARA (8mg) and thyme oil (6mg).

Summary of the Invention

A first aspect of the present invention relates to an edible formulation comprising ARA in an amount adapted to deliver a dosage (of ARA) of from 150mg to 1g per day.

Preferably the formulation is adapted to deliver from 200 to 900mg per day ARA, such as from 200 to 700mg per day, optimally from 250 to 400 or 500mg per day.

Edible formulations include dietary supplements and (pharmaceutical) formulations and preparations, such as tablets, pills and capsules. They additionally include (solid or liquid) foodstuffs, for example dairy products (margarine, butter, milk, yoghurt), bread, cakes; drinks such as beverages (tea, coffee, cocoa, chocolate drinks), fruit juices, soft (e.g. fizzy) drinks; confectionery; oily foods (snacks, salad dressing, mayonnaise), soups, sauces, carbohydrate-rich foods (rice, noodles, pasta), fish-containing foods, baby foods (such as infant formula, either as a liquid or powder), pet food, and ready prepared or microwaveable foods.

The ARA can be from any suitable source. It may be from a natural (e.g. vegetable or marine) source, or it may be from a microbial source or from a microorganism, such as fungus, bacterium or a yeast.

Suitable fungi are of the order *Mucorales*, for example *Mortierella*, *Pythium* or *Entomophthora*. The preferred source of ARA is from *Mortierella alpina or Pythium insidiosum*. Suitable commercially available ARA oils include those from DSM/Gist-brocades, Wateringseweg, P.O. Box 1, 2600 MA, Delft, The Netherlands under the trade mark OPTIMAR™ and from Martek Corporation, 6480 Dobbin Road, Columbia, MD 21045, USA, under the trade mark ARASCO™.

In addition to the ARA, one or more additional PUFAs may be provided. This may be another n-6 PUFA in addition to ARA (such as a C18, C20 or C22 fatty acid) or it may be a n-3 fatty acid (for example, a C18, C20 or C22 fatty acid) and in particular EPA and/or DHA. Each PUFA that may be used in the invention may be in the form

of a free fatty acid, fatty acid ester (e.g. methyl or ethyl ester) as a phospholipid or as a triglyceride.

If the formulation comprises an n-3 fatty acid, it is preferred that this is EPA or DHA. If it is DHA, then the formulation is preferably adapted to deliver the same dosage as specified for ARA, such as from 400 to 600mg per day DHA. Alternatively, or in addition, if the formulation comprises EPA, then it is preferably adapted to deliver a dosage of from 150mg to 1g per day EPA, such as from 250 to 500mg of EPA per day.

If the formulation is to be taken (eaten or ingested) once a day then it can contain from 150mg to lg of ARA. If twice a day then the formulation can have 75mg to 0.5g of ARA, for three times a day a content of 50mg to 330g ARA, and so on, pro rata, for more frequent administrations. The same calculations can be applicable for other PUFAs that may be present, such as DHA.

If the formulation comprises more than one PUFA then the amount of each PUFA can be expressed relatively, as a ratio. For example, if an n-3 PUFA is additionally provided, then the ratio of ARA:n-3 PUFA (such as DHA or EPA) can be from 1:5 to 5:1, preferably from 2:1 to 1:3, optimally from 1:1 to 1:2. The relative amounts of the PUFAs can be balanced so that PUFA levels are supplemented, increased (or at least not decreased significantly) bearing in mind the condition of the individual.

Preferably the PUFA is present in an oil. This may be a pure oil, a processed (e.g. chemically and/or enzymatically treated) or concentrated oil. This oil may comprise from 10 to 100% of the PUFA, but the content may be from 20 to 45%, optimally from 30 to 45% of the desired PUFA, for example ARA, if a microbial oil. Of course, this oil may contain one or more PUFAs within these percentage concentrations. The oil may be a single oil derived from a single cell or a microbial source, or it may be a blend or mixture of two or more oils from these or other (e.g. vegetable or marine) sources. The oil may contain one or more antioxidants (e.g. tocopherol, vitamin E, palmitate) for example at a concentration of from 50 to 800ppm, such as 100 to 700ppm. Suitable processes for preparing PUFAs are described in International patent application numbers PCT/EP97/01446 (WO-A-97/36996), PCT/EP97/01448 (WO-A-97/37032), and PCT/US92/00517 (WO-A-92/13086).

A second aspect of the invention relates to a (pharmaceutical) composition comprising ARA and DHA at a ratio of ARA:DHA of from 1:1 to 1:2. This ratio of PUFAs has been found to provide a good balance, and can increase *in vivo* DHA levels without ARA levels being suppressed due to a too high DHA content. The DHA can be

from a natural (e.g. marine) source or from a microbial source (e.g. from an algae).

A third aspect relates to an edible formulation (eg. a foodstuff) comprising from 0.1 to 3 or 5% ARA. Preferably, the amount is from 0.5 to 1.5 or 2%, optimally from 0.3 to 0.8%. Suitable foodstuffs have already been discussed in relation to the first aspect. Preferred methods of preparing infant formula are disclosed in International application numbers PCT/EP97/01447 (WO-A-97/35487) and PCT/EP97/01449 (WO-A-97/35488).

Suitable formulations can include oils, for example to be taken orally. The oil may be taken as such, or it may be encapsulated, for example in a shell, and may thus be in the form of capsules. The shell or capsules may comprise gelatin and/or glycerol. The formulation may contain other ingredients, for example flavourings (e.g. lemon or lime flavour).

The invention has found use in improving PUFA levels in normal, healthy, well fed individuals (who would normally not be expected to benefit if on an adequate diet). However it can also be used with individuals with low PUFA level(s) or deficiencies.

Thus, a fourth aspect of the present invention relates to the use of ARA (eg. as a dietary or nutritional supplement or for the manufacture of a medicament) for a woman who is:

a. pregnant and at an age of from 15 to 20;

b. pregnant and at an age of from 40 to 60, such as from 50 to 55;

c. pregnant with her fourth, fifth or subsequent child;

d. pregnant with twins, triplets or quadruplets;

e. pregnant and is from 1 to 3 months into her pregnancy;

f. pregnant as a result of *in vitro* fertilisation (IVF) or who is undergoing IVF treatment (which includes enrolling in or participating in an IVF procedure) but not yet pregnant;

g. pregnant at from 20 or more weeks of gestation;

h. pregnant and is malnourished, poorly or marginally nourished, suffering from malnutrition or malabsorption or deficient in one or more essential fatty acids (such as a PUFA);

i. trying to become pregnant;

j. pregnant, for promoting the intra-uterine growth or health of a foetus; or

k. lactating, for increasing the level of ARA or EPA in the woman's breast milk.

In the case of (h) these conditions are relatively rare in Western Europe, but may be

found in women in Africa or some Asian countries (eg. Pakistan).

For pregnant women, the benefit to the foetus in (j) has not always been predictable or immediately apparent due to the variance in individuals in the transport of fluid between the mother and foetus. The placenta to foetus connection (the umbilical cord) can vary in size and physiological condition and so in the past the supplementation of the mother with PUFAs has not necessarily indicated that the foetus will receive these PUFAs and so benefit also.

A fifth aspect relates to the use of ARA (as a dietary or nutritional supplement) for a human (male or female) over 50 years old, preferably over 65 years old.

A sixth aspect relates to the use of ARA (as a dietary or nutritional supplement) for a non-human mammal which is pregnant or lactating.

The ARA is preferably ingested at from 150 to 700mg per day, optimally from 250 to 500mg per day.

A seventh aspect of the present invention relates to the use of ARA for the manufacture of a medicament for (assisting in) the prophylaxis, prevention, amelioration or treatment of a disease or condition associated with an abnormal or low level of an n-3 or n-6 PUFA, for example in the blood. The invention therefore finds use in subjects that have low levels of ARA, for example for those that cannot or cannot effectively convert linoleic acid (LA) to ARA. Therefore, suitable patients may have a malfunctioning, inefficient or deficiency in Δ6-desaturase.

A (mouse) model of PUFA deficiency has been established and used to mimic the effects of malnourishment. This model has shown the beneficial effects of the formulations of the invention, including during pregnancy, for both the mother and foetus. It has also allowed simulation of poor placental transfer and intra-uterine growth retardation, and shown the benefits of supplementation with formulations of the invention in the individuals mentioned in the various aspects of the invention (and the foetus if pregnant).

The Applicant has found that certain diseases or conditions, in particular neuronal diseases, are associated with low levels of *in vivo* PUFAs, in particular low levels of ARA in the blood. It is therefore thought that the administration of ARA, or a balance of the PUFAs, will be able to assist in the prophylaxis, prevention, amelioration or treatment of these diseases or conditions. The diseases in question include: neuronal disease, such as schizophrenia, cystic fibrosis, idiopathic immunoglobulin A nephropathy, multiple sclerosis, retinitis pigmentosis, Usher's syndrome, celiac disease, macular degeneration,

Parkinsons' disease, osteoporosis, Alzheimer's disease or phenylketonuria.

An eighth aspect relates to the use of ARA, optionally with DHA, for promoting lactation and/or reproductive efficiency or success or fertility in a human or non-human female mammal.

A ninth aspect of the present invention relates to the use of ARA and DHA (in an edible formulation) at an ARA:DHA ratio that increases the ARA level in blood. Preferably the ratio of ARA:DHA is from 1:5 to 5:1, such as from 1:1 to 1:2.

The invention is particularly application to those people that have low ARA levels, for example a diabetic, alcoholic, drug abuser, smoker or a subject having an abnormal or low immune level or who is immunocompromised.

The use of the fourth to ninth aspects include methods of administration of the ARA (and optionally DHA), either as such or in a formulation, to a subject (individual, human or animal) where that subject is in need of, or will benefit from, the administration, or those uses in the manufacture of a medicament for the purposes specified. Formulations may exclude GLA and/or DGLA if necessary.

The dose or amount of ARA (and DHA, if present) is preferably such that it increases either an essential fatty acid (EFA) sufficiency index (defined as the level of 20:4 n-6 (ARA) divided by the level of 20:3 n-9 fatty acid (mead acid)) and/or an EFA balance index (defined as the level of 22:6 n-3 (DHA) divided by the level of 22:5 n-6). Here, levels include those in the blood (eg. in red blood cells), brain, placenta, liver, intestine, plasma or foetus.

Preferred features and characteristics of one aspect of the invention are equally applicable to another aspect *mutatis mutandis.*

The following Examples are provided to merely illustrate the invention, and are not to be construed to be limiting.

Examples 1 to 3: Preparation of a composition containing balanced proportions of PUFAs.

This example describes the blending of n-6 and n-3 oils so that they can be included in a single capsule.

The composition was prepared by combining one n-6 PUFA-rich oil with three different n-3 PUFA-rich oils. The n-6 PUFA-rich oil was derived from the fermentation of the filamentous fungus *Mortierella alpina*, and contained approximately 40% ARA as the major fatty acid. For the n-3 PUFA-rich oil the three different sources were: a

high-EPA (above 45%) low-DHA (about 10%) fish oil (from Pronova, Norway under the trade name EPAX™ , product no. EPAx4510TG), a high-DHA (above 50%) low-EPA (about 20%) fish oil (also from Pronova under the same brand name, product no. EPAx2050TG), and an oil derived from fermentation of the unicellular alga *Crypthecodinium cohnii* which contains 40% DHA as major fatty acid but is virtually devoid of EPA (from Martek Corporation, Columbia, United States of America under the trade name DHASCO™).

The oils were mixed in appropriate quantities to give the desired amounts and proportions of n-3 and n-6 PUFAs. Here the ARA:DHA ratio for the three blends (Examples 1 to 3) was 1:1. During this procedure, the oxidation-sensitive oils were protected from environmental oxygen by a blanket of oxygen-free nitrogen gas. Subsequently, the oils were used to prepare soft-gel gelatin capsules, where each capsule had 400mg ARA and 400mg DHA.

Example 4: Provision of balanced PUFAs to pregnant women during the early or latter stages of pregnancy.

This Example concerns the trial of pregnant women that are supplemented with ARA and DHA either between weeks 6 and 15 or between weeks 20 and 25 during pregnancy until delivery (birth). The ARA source was a triglyceride oil containing 38% ARA available from DSM/Gist-brocades, Delft, The Netherlands, under the trade name OPTIMAR™. This is an oil produced by the fungus *Mortierella alpina*. For DHA either a DHA-rich fish oil of food grade or an algae-derived oil obtained from Martek Corporation under the trade mark DHASCO™ was employed.

Maternal supplementation of ARA and DHA during pregnancy was therefore studied to see if the fatty acid status of the mother measured at birth and subsequently during lactation compared with the controlled group that received no supplementation. The measurements included maternal erythrocyte ARA and DHA values, ARA and DHA content of the umbilical arteries and venous vessel wall, ARA and DHA content of breast milk.

The study was a case controlled study involving 10 pregnant women. One experimental group (of five women) received one or more gelatin capsule (each of 250mg ARA) oil per day (containing 38% ARA) and one capsule (each of 500mg DHA) oil per day (containing 25% DHA). The control group received the same amount of placebo gelatin capsules to overcome differences in daily calorie intake. The vitamin E intake of

the experimental and controlled groups was equal, and the capsules were taken during breakfast.

Blood samples were taken at the beginning of the trial and at the end of gestation. Red blood cell fatty acids were measured (as phospholipids) using capillary gas chromatography with flame ionisation.

It was found that the supplemented women had significantly higher levels of both DHA and ARA in the red blood cells during pregnancy and at the time of birth. Remarkably, these higher levels persisted during the lactation period, being apparent both in the red blood cells of the mothers and their breast milk. The ARA level in breast milk was found to have risen to from 0.8 to 1.0% ARA. In addition the ARA levels in the blood of the newly born children was found to be higher than the control group. This finding is of major significance for mothers and their children under marginal nutritional conditions.

Example 5: Provision of balanced PUFAs to elderly people.

The Applicant perceives a need to enhance the n-3 PUFA status of the population, not in the least in the elderly population, where diseases such as Parkinson's disease and Alzheimer's disease have been found to be associated with a low PUFA status. This is thought to be partly due to inefficient or deficient Δ6-desaturase enzyme. However care is needed, especially in older people, since a decrease in ARA levels could exert a negative effect on the immune system.

A formulation was prepared according to Example 1, containing n-3 and n-6 PUFAs in a ratio of DHA:ARA of 2:1. The capsules were given to a group of healthy, elderly men and women (at least 65 years of age), at a dosage of 1 g n-3 PUFAs per day.

After one month the PUFA status of the red blood cells of the subjects was assessed. It was found that in all cases the levels of DHA had increased, whereas the levels of ARA had remained constant, or showed a slight increase in some cases. Thus it was possible to enhance the n-3 PUFA status of patients, without compromising the ARA status, by the use of a balanced formulation.

Example 6: Provision of PUFAs to pregnant women.

Two types of PUFA-containing capsules were prepared. The first contained ARA, at 500mg per capsule. These were to be taken one a day. The ARA was provided as a microbial oil, obtained from DSM/Gist-brocades, Delft, The Netherlands, under the

trade name OPTIMAR™. These capsules had a gelatin coat, and contained 20mg of vitamin E. Similar capsules were also prepared having the same amount (500mg) of DHA, being present as a microbial oil obtained from Martek Corporation, Columbia, United States of America (under the trade name DHASCO™). These capsules were also designed to be taken one per day.

Trials were conducted with pregnant women ingesting either one ARA capsule per day, or one ARA and one DHA capsule per day. The women chosen for the study were those that had been found to have relatively low levels of ARA in the blood. A number of women who were pregnant were therefore tested for *in vivo* ARA blood levels and permission was obtained to take part in the study. The first group of women were teenagers of from 15 to 20 years of age. For all these women, this was their first pregnancy. Due to early maturation they were found to benefit from both ARA and ARA plus DHA supplementation in their diet. Both regimes increased *in vivo* ARA levels.

A second group of women, also pregnant, were studied, these being from age 40 to 50. During pregnancy it was also found their *in vivo* blood levels were increasing under both supplementation regimes. Half of the women chosen in this study were having their fourth child.

Three women each pregnant with twins were chosen for supplementation with one ARA capsule and one DHA capsule per day. Their ARA *in vivo* levels were found to be relatively low, probably because the ARA from the blood of the mother was being absorbed and consumed by both foetuses. These women were supplemented with the ARA and DHA capsules and the ARA levels in the blood were found to increase.

Example 7: Provision of ARA and DHA to subjects with low PUFA content.

The same capsules were used as described in Example 6, except this time the ARA capsules contained only 250mg ARA. These capsules could be taken once or twice daily, according to the subject and their condition.

A number of people were chosen for this study due to their relatively low content of PUFAs in the blood. The reason for the low PUFA content was not always immediately evident. However, it has been found that a number of diseases or adverse conditions lead to low PUFA levels, and it was therefore postulated that providing either a correct dosage of ARA, or a balance of ARA:DHA, the *in vivo* ARA levels could be increased, which might moderate some of the symptoms of the condition. Some of the

conditions were thought to result in a poor efficiency in conversion of a precursor to ARA itself, for example a defect or deficiency with the enzyme Δ6-desaturase. Those conditions that were found by the Applicant to often give rise to low PUFA levels included cystic fibrosis, multiple sclerosis, celiac disease and osteoporosis. In addition, patients who were being treated for alcoholism, addiction to drugs or who were immunocompromised (AIDs patients) were also found to have low levels of PUFAs.

A study was therefore made where either one or two ARA capsules were taken daily, to give an ARA:DHA content of either 1:1 or 1:2. In almost all cases those subjects who were taking these capsules (for at least 3 weeks) were all found to have, at the end of the trial, increased *in vivo* ARA blood levels.

Example 8: Provision of PUFAs in infant formula.

Both solid (powdered) and liquid infant formula baby food was prepared containing 0.5% ARA and 0.5% DHA. This formula was fed to babies regularly in their first three months by mothers who had decided not to breast feed their children. As a control, the *in vivo* ARA blood levels of these children were compared to those that were being breast fed over the same time period. It was found that in the infants being bottle fed that their ARA levels were comparable to those being breast fed.

Comparative Example 9 and Example 10

A number of breast feeding women were chosen for a comparative trial. One group of women were fed two EFANATAL™ capsules per day (to give a daily intake of DHA 125mg, ARA 8.6mg and GLA 40mg). For comparison, a second group of women were given similarly prepared capsules (with a gelatin/glycerol shell) containing 150mg ARA per capsules (to give a daily ARA intake of 300mg ARA,2 capsules per day). In this second group a third capsule was also taken, one per day, which contained DHA at 500mg per capsule.

The ARA levels in the lactating women in both groups, after child birth, was compared. Also compared was the level of ARA in the mothers breast milk.

In the first EFANATAL™ group the ARA levels were found to have decreased markedly in the blood, and to a lesser extent in the breast milk, only two weeks after the trial involving consumption of EFANATAL™ had begun. In contrast those women taking the two capsules of ARA and one capsule of DHA per day were found to have the ARA levels in their blood increase, and the breast milk levels also increased to above

0.7%.

Example 11: Amelioration of fatty acid deficiency in mouse pregnancy through supplementation with ARA and DHA.

A major problem during the pregnancy of humans and non-human mammals is the occurrence of intra-uterine growth retardation. This condition is associated with significant health risks for the infant after birth that may continue into adult life. The condition can develop even during pregnancy of an apparently healthy woman and is difficult to predict. It is generally assumed that it is caused by poor functioning of the placental interchange, for instance because the placenta is too small or in poor physiological condition.

This unpredictability has obstructed the development of a reliable animal model for this condition. In principle one could simulate a poor placental function by decreasing the blood flow through the umbilical vein, for instance by restricting its diameter by a clamp. The problem with this method is that it requires surgery of the pregnant animal, which can adversely affect both the foetus and the mother, and it is difficult to achieve a uniform decrease of the blood flow in this way. Therefore a different model has been developed. A poor placental function translates into a decreased supply of essential fatty acids (EFAs) to the foetus. In the 'natural' condition this is caused by a decreased blood flow, at an otherwise normal physiological concentration in the blood of the healthy mother. In the present example we have simulated this condition by decreasing the concentration of the essential fatty acids in the blood of the mother, but having a normal flow through the placenta. For this purpose an early phase of fatty acid deficiency in pregnant mice was induced. In this phase the deficiency was expressed in biochemical parameters, but functional defects were not apparent. Thus it was ensured that while the pregnancy proceeded in the normal way the supply of essential fatty acids to the foetus was restricted.

In the trial 40 female mice, 8-10 weeks of age, were fed a regular mouse chow diet for 1 week. Subsequently they were divided into 8 experimental groups: RD 1 to 4 and EFAD 1 to 4. The RD groups continued to receive a regular chow diet, containing 6.5% of fat. The EFAD groups received an essential fatty acid deficient diet. The numbers 1 to 4 indicate various lipid supplements, according to Table 1. ARA was from DSM, Delft, and DHA from Pronova (fish oil) as described in previous Examples.

Table 1: Amounts of lipid supplements as percentage of total dietary lipids. The diets contained between 3.8% of 5.6% (g/g) lipids.

RD or EFAD	MCT (Medium-Chain Triglycerides)	ARA (Arachidonic Acid Oil)	DHA (Docosahexaenoic Acid Oil)
1	19	0	0
2	15	4	0
3	4	0	15
4	0	4	15

The fatty acid composition of the RD (regular diet) and the EFAD (essential fatty acid deficient) diets as well as the oil supplements are given in Table 2.

Table 2: Fatty acid composition of lipid fractions, expressed as g% of total fatty acids.

Fatty Acid	RD lipid	EFAD lipid	MCT	ARA oil	DHA oil
8:0-12:0			100.00		
14:0	0.10			1.90	3.60
16:0	10.00	44.78		16.14	19.50
17:0	0.10				
18:0	4.00	54.73		12.10	5.11
20:0	0.30			0.85	0.34
22:0	0.30			1.48	0.29
24:0	0.20			1.55	0.18
18:3ω3	7.50				0.58
18:4ω3					0.96
20:4ω3					0.39
20:5ω3					6.52
22:5ω3					1.33
22:6ω3(DHA)					25.08
18:2ω6	55.00			7.01	1.74
18:3ω6				3.24	0.20

20:2ω6				0.38	0.30
20:3ω6				3.85	0.11
20:4ω6 (ARA)				37.64	2.15
22:4ω6					0.41
22:5ω6					8.32
16:1ω7					6.00
18:1ω7				0.45	2.77
18:1ω9	22.50	0.50		13.01	12.60
20:1ω9				0.36	0.96
22:1ω9					0.12
20:3ω9				0.04	
24:1ω9					0.46

Two additional control groups were included. One group (RD 0) did not receive any lipid supplement. The second group received the same diet as RD 0, but served as a non-pregnant (NP) outgroup. The animals had unrestricted access to the diets.

The experimental groups were treated according to the time schedule shown below.

Table 3: Time schedule of treatments.

Day	Treatment
day - 3	Intraperitoneal injection of 5 IU Folligonan (FSH) IP (all groups except NP). Regular diet replaced by experimental diets
day - 1	Intraperitoneal injection of 5 IU Chorulon (hHCG) IP (all groups except NP). Male mice introduced into the cages (all groups except NP)
day 0	Males removed
day 15	Animals killed by heart puncture under halothane anaesthesia (4-6% in N_2O/O_2

The hormone treatment with Folligonan™ and Chorulon™ (from Organon, the Netherlands) induced super-ovulation in the females. This procedure, combined with the short exposure to the males, gave a reasonable probability of pregnancy, but no guarantee. The fatty acid composition of various tissues or sections of both the pregnant

mice and their foetuses was determined by gas chromatography. The fractionation, homogenisation and extraction of the various tissues was performed by methods known in the art.

On average, the animals consumed 3.9g of the diets per day, without significant differences between the various RD and EFAD groups. The dietary dosage of PUFAs is shown in Table 4.

Table 4: Dietary dosage of ARA and DHA, expressed as a percentage of the lipid fraction and as mg intake per day.

No.	Diet	ARA		DHA	
		% of lipid	mg/day	% of lipid	mg/day
0	RD	0	0	0	0
1	RD+MCT	0	0	0	0
2	RD+ARA/MCT	1.29	2.7	0	0
3	RD+DHA/MCT	0.30	0.5	3.30	5.1
4	RD+ARA/DHA	1.63	2.5	3.25	5.0
1	EFAD+MCT	0	0	0	0
2	EFAD+ARA/MCT	1.11	2.4	0	0
3	EFAD+DHA/MCT	0.34	0.5	3.73	5.9
4	EFAD+ARA/DHA	1.58	2.3	3.27	4.8

First it was checked whether the EFAD indeed induced a biochemically relevant essential fatty acid deficiency in the blood of the female mice. There were few differences in the blood levels of various fatty acids between pregnant and non-pregnant mice of the same dietary group as seen in the comparison with RD0 and NP (data not shown). Therefore these two groups were compared, to increase the statistical power of the comparison, except in the cases where there was a significant difference between pregnant and non-pregnant animals. In those cases, the values for the pregnant individuals was used. The results are shown in Table 5.

Table 5: Levels of essential fatty acids (EFAs) in red blood cells of female mice.

PUFA (ratio)	RD+MCT	EFAD+MCT
18:3 n-3	0.19 ± 0.02	0.05 ± 0.01
20:5 n-3	0.24 ± 0.02	0.09 ± 0.03
22:6 n-3 (DHA)	6.38 ± 0.25	4.53 ± 0.28
18:2 n-6	8.43 ± 0.08	3.00 ± 0.12
20:4 n-6 (ARA)	17.23 ± 0.44	18.87 ± 0.85
EFA sufficiency index: 20:4 n-6/20:3 n-9	63	11
EFA balance index: 22:6 n-3/ 22:5 n-6	11	4

The EFAD caused a marked decrease in the level of essential fatty acids, with the exception of arachidonic acid. However, in spite of the maintenance of the level of arachidonic acid, there was a marked (n-6) essential fatty acid deficiency. This is clearly seen in the EFA sufficiency index, the ratio between the level of arachidonic acid (20:4 n-6) and its non-essential analogue mead acid (20:3 n-9). This latter fatty acid accumulates only if there are insufficient essential fatty acids as substrates for normal biosynthesis: in that case the non-essential fatty acid oleic acid (18:1 n-9) is elongated and desaturated instead, leading to the formation of n-9 analogues of the physiological PUFAs. It is clear from Table 5 that this EFA sufficiency index dropped dramatically in the EFAD-fed mice.

Another index indicates the correct balance of n-3 and n-6 essential fatty acids. This EFA balance index is the ratio between DHA (22:6 n-3) and arachidonic acid (22:4 n-6). This index also strongly decreased in the EFAD group.

So these data shown that the EFAD diet indeed induced a clear biochemical EFA deficiency, as was intended.

It was then checked whether the addition of arachidonic acid and/or DHA to the diet would lead to alleviation of this deficiency in the red blood cells of the female mice. First the control data of the fatty acid sufficient (RD) mice are presented in Table 6.

Table 6: Effect of PUFA supplementation on essential fatty acids in red blood cells of fatty acid-sufficient female mice. Fatty acid data expressed as percentage of the RD-group.

	RD	RD+ARA/MCT	RD+DHA/MCT	RD+ARA/DHA
18:3 n-3	100%	93%	74%	82%
22:6 n-3 (DHA)	100%	85%	131%	132%
18:2 n-6	100%	84%	94%	100%
20:4 n-6 (ARA)	100%	107%	79%	93%
20:4 n-6/20:3 n-9	63	59	59	67
22:6 n-3/22:5 n-6	11	9	15	14

It was found that addition of the supplements with either ARA or DHA depressed the levels of the other PUFA. In contrast, the combined supplement allowed the enhancement of the PUFA status, even in fatty acid sufficient mice. The supplement used caused a slight depression of the ARA status, causing an increase of the EFA balance index. This could be due to the ratio chosen, with DHA:ARA approximately at 2:1. Surprisingly, the EFA-sufficiency index was also enhanced by the supplement, even though these mice were apparently not fatty acid deficient.

It was then investigated whether the supplementation with PUFAs led to an improvement in the essential fatty acid status in the blood cells of the EFAD-fed animals.

Table 7: Effect of PUFA supplementation on essential fatty acids in red blood cells of fatty acid-deficient female mice. Fatty acid data are expressed as a percentage of the RD-group.

	EFAD	EFAD+ARA/MCT	EFAD+ DHA/MCT	EFAD+ ARA/DHA
18:3 n-3	24%	37%	25%	34%
22:6 n-3 (DHA)	71%	70%	174%	171%
18:2 n-6	36%	42%	49%	44%
20:4 n-6 (ARA)	109%	121%	70%	90%
20:4 n-6/20:3 n-9	11	43	43	67
22:6 n-3/22:5 n-6	4	5	16	16

Table 7 shows that the EFAD-mice responded quite strongly to the PUFA-supplement, especially in their DHA status. While there are no indications that supplementation with ARA depresses the DHA status, the converse is clearly true: the addition of the DHA supplement caused a clear depression of the ARA status. It is also clear that the addition of PUFAs specifically restored PUFA levels, with the levels of the C-18 fatty acids being much less affected. Interestingly, the combined supplement was the only one that caused full restoration of the EFA sufficiency index.

Finally it was investigated whether the enhancement of the PUFA status in the blood of the mother would lead to an improved status of the fetus. To this end we chose the head of the foetus as the most relevant compartment: the growth of the brain (and other neural tissue) is quantitatively the most important process depending on the provision of PUFAs.

The data for the foetuses of the RD-fed mothers are shown in Table 8.

Table 8: Effect of PUFA supplementation of EFA-sufficient mothers on essential fatty acids in mice foetus heads. Fatty acid data in the RD-group is expressed as mol-percent. Fatty acid data for the experimental groups is expressed as percentage of the RD-group.

	RD	RD+ARA/MCT	RD+DHA/MCT	RD+ARA/DHA
22:6 n-3 (DHA	5.89	101%	135%	125%
20:4 n-6 (ARA)	11.87	103%	93%	102%
20:4 n-6/20:3 n-9	27	30	28	40
22:6 n-3/22:5 n-6	8	6	18	13

The data show that the supplements caused modest changes in the concentrations of PUFAs in the heads of foetuses of the RD-fed mice. Surprisingly, there was a marked improvement in the EFA sufficiency index for the combined supplement, as opposed to the separate supplements. In addition, both DHA-containing supplements caused a significant increase in the EFA balance index.

Table 9: Effect of PUFA supplementation of EFA-deficient mothers on essential fatty acids in mouse foetus heads. Fatty acid data expressed as percentage of the RD-group. The EFAD+AA/MCT group did not contain pregnant females.

	EFAD	EFAD+ARA/MCT	EFAD+DHA/ MCT	EFAD+ARA/ DHA
22:6 n-3 (DHA)	61%	-	160%	146%
20:4 n-6 (ARA)	98%	-	76%	83%
20:4 n-6/20:3 n-9	9	-	14	17
20:6 n-3/22:5 n-6	2	-	33	24

The fatty acid deficiency of the foetuses was even more severe than that of the mothers. The PUFA-supplements led to a marked improvement of the EFA sufficiency index, almost restored to the RD-level. This was probably due to the relatively low dosage of arachidonic acid in the supplement, since the EFA balance index is even higher than in the foetuses of the RD-fed mothers. This implies that the PUFAs are efficiently incorporated into the foetus head. Indeed the inclusion of arachidonic acid in the supplement increases its concentration, although not up to the RD- level. This emphasises the need to balance the supplementation. The appropriate balance can then be assessed experimentally.

CLAIMS

1. An edible formulation comprising arachidonic acid (ARA) in an amount adapted to deliver a dosage of from 150mg to 1g/day ARA.

2. A formulation according to claim 1 which is adapted to deliver from 250 to 500 mg/day ARA.

3. A formulation according to claim 1 to 2 which is additionally adapted to deliver docosahexaenoic acid (DHA).

4. A formulation according to any preceding claim which is adapted to deliver a dosage of from 400 to 600 mg/day DHA.

5. A formulation according to any preceding claim wherein the ratio of ARA:DHA is from 1:5 to 5:1, such as from 1:1 to 1:2.

6. An edible formulation comprising from 150 to 700 mg ARA which is intended to be ingested once per day.

7. An edible formulation comprising from 75 to 350 mg ARA which is adapted to be ingested twice per day.

8. An edible formulation according to any preceding claim which is a food or nutritional supplement.

9. An edible formulation according to any preceding claim which is a pharmaceutical composition.

10. A pharmaceutical composition comprising ARA and DHA at a ratio of ARA:DHA at from 1:1 to 1:2.

11. A foodstuff comprising from 0.1 to 5% ARA.

12. The use of ARA as a dietary or nutritional supplement for a woman who is:

a. pregnant and at an age of from 15 to 20;

b. pregnant and at an age of from 40 to 60, such as from 50 to 55;

c. pregnant with her fourth, fifth or subsequent child;

d. pregnant with twins, triplets or quadruplets;

e. pregnant and is from 1 to 3 months into her pregnancy;

f. pregnant as a result of in vitro fertilisation (IVF) or who is undergoing IVF treatment but not yet pregnant;

g. pregnant at from 20 or more weeks of gestation;

h. pregnant and is malnourished, poorly or marginally nourished, suffering

from malnutrition or malabsorption or deficient in one or more essential fatty acids;

i. trying to become pregnant;

j. pregnant, for promoting the intra-uterine growth of health of a foetus; or

k. lactating, for increasing the level of ARA or EPA in the woman's breast milk.

13. The use according to claim 12 wherein the ARA is ingested at from 150 to 700, such as from 250 to 500, mg/day.

14. The use of ARA as a dietary or nutritional supplement for a human who is over 50 years old, preferably over 65 years old.

15. The use of ARA as a dietary or nutritional supplement for a non-human mammal which is pregnant or lactating.

16. The use of ARA for the manufacture of a medicament for assisting in the prophylaxis, prevention, amelioration or treatment of a disease or condition associated with an abnormal or low level of an n-3 or n-6 PUFA in the blood.

17. The use according to claim 16 wherein the disease or condition is a neuronal disease, such as schizophrenia, cystic fibrosis, idiopathic immunoglobulin A nephropathy, multiple sclerosis, retinitis pigmentosis, Usher's syndrome, celiac disease, macular degeneration, Parkinsons' disease, osteoporosis, Alzheimer's disease or phenylketonuria.

18. The use of ARA for promoting lactation and/or reproductive efficiency or success, or fertility in a human or non-human female mammal.

19. The use of ARA and DHA in an edible formulation at an ARA:DHA ratio that increases the ARA level in blood.

20. The use according to claim 19 wherein the ratio of ARA:DHA is from 1:5 to 5:1.

21. The use according to claim 20 wherein the ratio of ARA:DHA is from 1:1 to 1:2.

22. The use according to any of claims 19 to 21 for a person who is a diabetic, an alcoholic, a drug abuser, smoker or who is immunocompromised or has an abnormal immune level.

INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/20 A23L1/30 A23K1/16

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B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K A23L A23K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Caturla Vicente, V

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(71) Applicant *(for all designated States except US)*: DSM N.V. [NL/NL]; Het Overloon 1, NL–6411 TE Heerlen (NL).

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(54) Title: PUFA SUPPLEMENTS

(57) Abstract

Edible formulations, such as polyunsaturated fatty acids (PUFAs) such as pharmaceutical compositions or nutritional supplements, are disclosed comprising arachidonic acid (ARA). They are adapted to deliver from 150 mg to 1 g per day of ARA and may contain other PUFAs, for example docosahexaenoic acid (DHA). The DHA dosage is from 400 to 600 mg per day, and the ratio of ARA:DHA may be from 1:5 to 5:1. Pharmaceutical compositions comprising ARA and DHA at a ratio of ARA:DHA of 1:1 to 1:2 are also disclosed, as are foodstuffs comprising 0.1 to 5 % ARA. Such formulations can be used to increase ARA levels *in vivo*, for example in pregnant women or for people who have diseases or conditions associated with low ARA levels.

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INTERNATIONAL SEARCH REPORT

International Application No
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